JOURNAL OF CLINICAL MICROBIOLOGY, July 2005, p. 3484–3486 Vol. 43, No. 7
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.7.3484–3486.2005

Frequent Detection of Epstein-Barr Virus and Cytomegalovirus but Not JC Virus DNA in Cerebrospinal Fluid Samples from Human Immunodeficiency Virus-Infected Patients in Northern Thailand

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Received 18 August 2004/Returned for modification 18 October 2004/Accepted 27 February 2005

Applying nested-PCRs, we frequently detected DNA of Epstein-Barr virus and cytomegalovirus but not JC virus in cerebrospinal fluid samples from 140 human immunodeficiency virus-infected patients with central nervous system symptoms in northern Thailand. Despite the low incidence of primary central nervous system lymphoma or cytomegalovirus encephalitis among Thai AIDS patients, Epstein-Barr virus and cytomegalovirus infections in the central nervous system are common.

According to reports from the Thai Ministry of Public Health, opportunistic infections are common in the central nervous system (CNS) of Thai AIDS patients and have caused a significant portion of mortality. Cryptococcal meningitis was noted as 20.3% of the first AIDS defining illness in northern Thailand; toxoplasma encephalitis was 5.3%; tuberculous meningitis was also seen, though the exact prevalence in the total number of AIDS patients is unknown (2). Virus infections in the CNS such as Epstein-Barr virus (EBV), cytomegalovirus (CMV), and JC virus (JCV) can result in life-threatening consequences as they cause primary CNS lymphoma, cytomegalovirus encephalitis, and progressive multifocal leukoencephalopathy, respectively. In developed countries, PCR tests to detect EBV, CMV and JCV DNA in the cerebrospinal fluid (CSF) have been used as a supplemental diagnostic test (5). However, in developing countries, such a test is not available and very limited data have been reported about the prevalence of virus infections in the CNS. The objective of this study is to investigate the significance of EBV, CMV, and JCV infections in the CNS of human immunodeficiency virus (HIV)-infected Thais in northern Thailand.

From March 2001 to June 2003, CSF samples of 140 HIV-1-infected patients at the day care center clinic or the HIV/AIDS ward in Lampang Hospital, which is a Thai government referral hospital for Lampang province in northern Thailand, were examined as they were clinically suspected of having opportunistic infections in the CNS and did not have any contraindication for lumbar puncture. Consequently, 163 CSF samples including follow-up CSF samples were taken. All CSF samples were initially examined for routine laboratory tests such as cell count, protein concentration, sugar level, bacterial and fungal culture, Indian ink stain, Gram stain, acid-fast bacilli stain, and a latex agglutination test for cryptococcal antigen (PASTOREX, Bio-Rad, France). After the routine laboratory tests, residual CSF samples were stored at −80°C until DNA extraction.

All study patients gave informed consent when they participated in the Lampang HIV cohort study, which was approved by the Thai government ethics committee. DNA was extracted from 200 μl of CSF (QIAGEN blood mini DNA extraction kit, QIAGEN, California), eluted with 50 μl of distilled water, and 10 ul were used as the target for PCR. PCR amplifications were performed using ExTaq DNA polymerase (TaKaRa Biomedical, Osaka, Japan) and nested primer sets targeting specific sequences of virus genes as previously published: the EBNA-1 gene for EBV (PCR product, 209 bp) (3), immediately early protein gene for CMV (146 bp) (1), and regulatory regions for JCV (approximately 396 bp) (7, 10).

Diagnosis of EBV and CMV infection was made on the basis of the size of amplicons, but for the diagnosis of JCV, we further sequenced PCR products. The positive control for EBV PCR was DNA extracted from Namalwa cells as previously described (11). DNA extract from culture supernatant of CMV-producing fibroblast cells was used as a positive control for CMV PCR. DNA extract from the urine of a healthy JCV carrier was used as a positive control for JCV PCR. The detection limit of nested PCR for EBV and CJV was evaluated as previously described (10, 11). The detection limit of CMV PCR was approximately 100 copies/ml of CSF, which was estimated by a limiting dilution method using a DNA sample, of which the number of CMV copies was determined by a quantitative real-time PCR (Mitsubishi-Kagaku BCL, Tokyo, Japan).

The median (interquartile range; range) of age among 140 patients was 33 years (30 to 37 years; 20 to 63 years); 93 patients (66.4%) were male. $CD4^+$ T-cell count data were

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TABLE 1. Clinical characteristics of study patients[a]

Parameter	No. of patients (%)
Symptoms	
Altered mental status	37 (27.0)
Focal sign	18 (13.1)
Chronic headache	121 (88.3)
Fever	104 (75.9)
Diagnosis of CNS infection	
Cryptococcal meningitis[b]	98 (70.0)
Toxoplasmic encephalitis[b]	10 (7.1)
Tuberculous meningitis	6 (4.3)
Aseptic meningitis	3 (2.1)
No diagnosis	24 (17.1)
Antiretroviral drug therapy	
None	131 (93.6)
Two drugs	3 (2.1)
Three drugs	4 (2.9)
Unknown	2 (1.4)
Status at discharge	
Improved	99 (70.7)
Dead	29 (20.7)
Referred to another hospital	7 (5.0)
Unknown	5 (3.6)

[a] Medical records were available for 137 patients.
[b] Includes one case with both cryptococcal meningitis and toxoplasmic encephalitis.

available in 48 patients; the median (IQR; range) was 16 (7 to 42/μl; 0 to 605/μl). Clinical pictures of the patients are summarized in Table 1. Cryptococcal meningitis was by far the most common opportunistic infection in the CNS. There was no case of primary CNS lymphoma, CMV encephalitis, or progressive multifocal leukoencephalopathy. However, one patient developed clinical symptoms of progressive multifocal leukoencephalopathy during the follow-up period.

Thirty-one of 140 patients (22.1%) were positive for EBV PCR and 16 of 140 (11.4%) patients were positive for CMV PCR. Six patients were positive for both EBV and CMV PCR. More than one CSF sample was collected from 20 patients. The results of CMV PCR were concordant in all pairs of samples, but the results of EBV PCR were discordant in five pairs. None of the 140 first CSF samples was positive for JCV PCR. However, JCV was detected in the second CSF sample of one cryptococcal meningitis case. We found that patients with EBV DNA in the CSF tended to be older than the other patients and had a significantly higher protein concentration and a higher number of cells in the CSF (Table 2). We did not find any factor significantly associated with CMV DNA detection in CSF.

We found that EBV infection in the CNS is common in advanced HIV-infected patients in northern Thailand. This frequency was higher than the result of a similar study in Italy (4). The majority of our study patients were suffering from cryptococcal meningitis, but the detection rate of EBV DNA did not significantly differ according to the clinical diagnosis of cryptococcal meningitis. A significant association of EBV detection with a CSF cell count raised the concern that we may have detected EBV in the lymphocytes circulating in the peripheral blood, which invaded the CSF, rather than EBV of the CNS involvement. However, EBV was also often detected in patients without a CSF cell: EBV DNA was detected in CSF from 9 (19.6%) of 46 patients with a CSF cell count of 0.

Several studies from Western countries have shown a high sensitivity and specificity of EBV PCR in CSF for diagnosing primary CNS lymphoma (5). However, we have not seen any primary CNS lymphoma cases in our experience of having seen over 2,400 HIV-1-infected patients at the day care center clinic from its establishment on October 1995 to July 2004. Furthermore, the government report of adult AIDS patients from 1994 to 1998 showed that there were 98 primary CNS lymphoma cases, which represented only 0.1% of all reported first AIDS-defining illness in Thailand (2). According to the Thai national

TABLE 2. Factors associated with EBV or CMV DNA detection in the CSF[a]

Parameter	Median no. of patients (IQR)					
	EBV			CMV		
	PCR positive (n = 31)	PCR negative (n = 109)	P	PCR positive (n = 16)	PCR negative (n = 124)	P
Age (yr)	35 (31–42)	33 (30–36)	0.069	32 (29–38)	33 (30–37)	0.7
No. female	11 (35.5%)	36 (33.1%)	0.79	6 (37.5%)	41 (33.1%)	0.72
CSF cell count (/μl)	8 (0–66)	4 (0–10)	0.045	6 (0–18)	4 (0–16)	0.97
CSF protein concn (mg/dl)	80 (55–160)	53 (34–90)	0.003	75 (30–88)	57.5 (40–100)	0.96
Clinical diagnosis[b]						
Cryptococcal meningitis	21 (70.0%)	76 (69.7%)		12 (75.0%)	85 (69.1%)	
Toxoplasmisc encephalitis	4 (13.3%)	5 (4.6%)	0.40	1 (6.3%)	8 (6.5%)	0.65
Tubercular meningitis	1 (3.3%)	5 (4.6%)		0 (0.0%)	6 (4.9%)	
Aseptic meningitis	0 (0.0%)	3 (2.8)		1 (6.3%)	2 (1.6%)	
No apparent CNS infection	4 (13.3%)	20 (18.3%)		2 (12.5%)	22 (17.9%)	
Symptoms[c]						
Altered mental status	12 (40.0%)	25 (23.4%)	0.07	4 (25.0%)	33 (27.3%)	0.85
Headache	27 (90.0%)	94 (87.9%)	0.75	15 (93.8%)	106 (87.6%)	0.47
Focal sign	4 (13.3%)	14 (13.1%)	0.97	1 (6.3%)	17 (14.0%)	0.34
Fever	22 (73.3%)	82 (76.6%)	0.71	11 (68.8%)	93 (76.9%)	0.48
Death at discharge[d]	5 (17.2%)	24 (24.2%)	0.43	4 (25.0%)	25 (22.3%)	0.81

[a] Data are median (interquartile range) or number of patients (%).
[b] One case with cryptococcal meningitis and toxoplasmic encephalitis was excluded from the analysis.
[c] Medical records were available for 137 patients.
[d] Survival status at discharge was known for 128 patients.

guideline for clinical management of HIV/AIDS patients (8), if patients with a focal sign have poor response to the toxoplasma encephalitis therapy, further investigation with computed tomography scan is recommended to exclude other space-occupying lesions such as primary CNS lymphoma, and the computed tomography scan is available at most government referral hospitals in Thailand. However, this clinical practice may underdiagnose a minimal primary CNS lymphoma, which does not cause CNS symptoms.

Because of a high mortality rate of symptomatic Thai patients (9), patients with a small primary CNS lymphoma might have died due to other opportunistic infections before the primary CNS lymphoma lesion became large and caused CNS symptoms. Recently the Thai government pharmaceutical organization has started mass production of generic antiretroviral drugs. If many insidious primary CNS lymphoma cases exist in Thailand, we expect to see more patients with apparent primary CNS lymphoma lesions as the antiretroviral drug-treated patients survive longer. Alternatively, it is plausible that Thai patients are less susceptible to the development of primary CNS lymphoma and that EBV DNA detection in CSF from AIDS patients does not supplement the diagnosis of primary CNS lymphoma in Thailand.

In our experiences at Lampang Hospital, CMV retinitis is common among our advanced HIV-infected patients, but we have not seen any case with CMV encephalitis. This rarity of CMV encephalitis may be due to the difficulty of making a firm diagnosis in Thailand, since it requires magnetic resonance imaging or biopsy, which is not widely available, and the disease does not induce characteristic clinical symptoms. Our data on CMV PCR warn that we may be overlooking patients with CMV encephalitis.

Progressive multifocal leukoencephalopathy cases have been reported but are not common in Thailand (2, 6). At Lampang Hospital, we had one male patient who presented with hemiparesis and was diagnosed with progressive multifocal leukoencephalopathy on the basis of computed tomography scan findings and clinical course. His CSF was negative for JCV PCR, but this result does not exclude progressive multifocal leukoencephalopathy as the sensitivity of JCV PCR is not high (5). We found one case in which JCV virus was detected in the CSF of the second lumbar puncture. This patient did not have any other CNS symptoms besides headache, but he died shortly after the diagnosis of cryptococcal meningitis. We think that a low prevalence of JCV DNA detection is compatible with our clinical impression, that is, progressive multifocal leukoencephalopathy cases are there but not common, though more patients would be detected if brain magnetic resonance imaging were available.

We thank Noriaki Hosoya, Mieko Goto, and Tadashi Narisawa for their technical assistance and for Suthiraa Kasemsuk, Sriprai Seneewong-na-ayoottaya, Kethkaew Thamachai, Somchai Niyomthai, Anong-nard Ariyakruea, Nutira Boona, and Prapan Wongnamnong for their support.

This study was supported by the Japan International Cooperation Agency (JICA), the Ministry of Health, Labor and Social Welfare of Japan, and the Ministry of Public Health of Thailand.

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